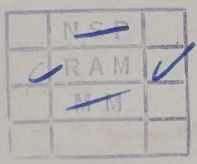
THE WHITE-RUST DISEASE OF HORSERADISH

By R. M. ENDO and M. B. LINN



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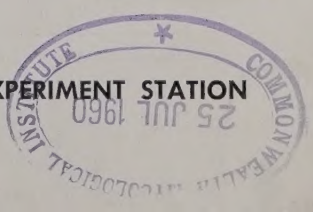

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THE WHITE-RUST DISEASE OF HORSERADISH

R. M. Endo[1] and M. B. Linn[2]

A LARGE PORTION OF THE HORSERADISH[3] GROWN IN the United States is raised in Illinois. Its most damaging foliage disease is white rust, which is caused by the fungus pathogen, *Albugo candida* (Pers. ex Chev.) Kuntze. The extensive leaf damage due to white rust prevents normal root growth and hence results in severe crop losses. The fungus is also believed to cause a crown, or head, rot of the root.

This bulletin is the result of a study of the factors governing the germination of the fungus, the initiation and development of the disease, and the various methods by which the fungus may overwinter. On the basis of these studies, several recommendations for control of the white-rust disease have been proposed.

SYMPTOMS

White rust can be recognized by the characteristic white pustules or sori which appear most often on the leaf blades (Fig. 1) and occasionally on the petioles (Fig. 2). The initial symptoms consist of small chlorotic areas which appear on both leaf surfaces but are more distinct on the upper surface. The sori soon appear within the chlorotic areas, most frequently on the underside of the leaf and only rarely on the upper. They are at first small, flat, and grayish white but later become raised and creamy white. The sori vary considerably in shape but are usually oval, irregularly oval, or elongated, and range in diameter from 0.5 mm. to 8 mm. Eventually the pressure exerted by the growing fungus ruptures the epidermis of the leaf. The sori may then appear powdery and grayish white.

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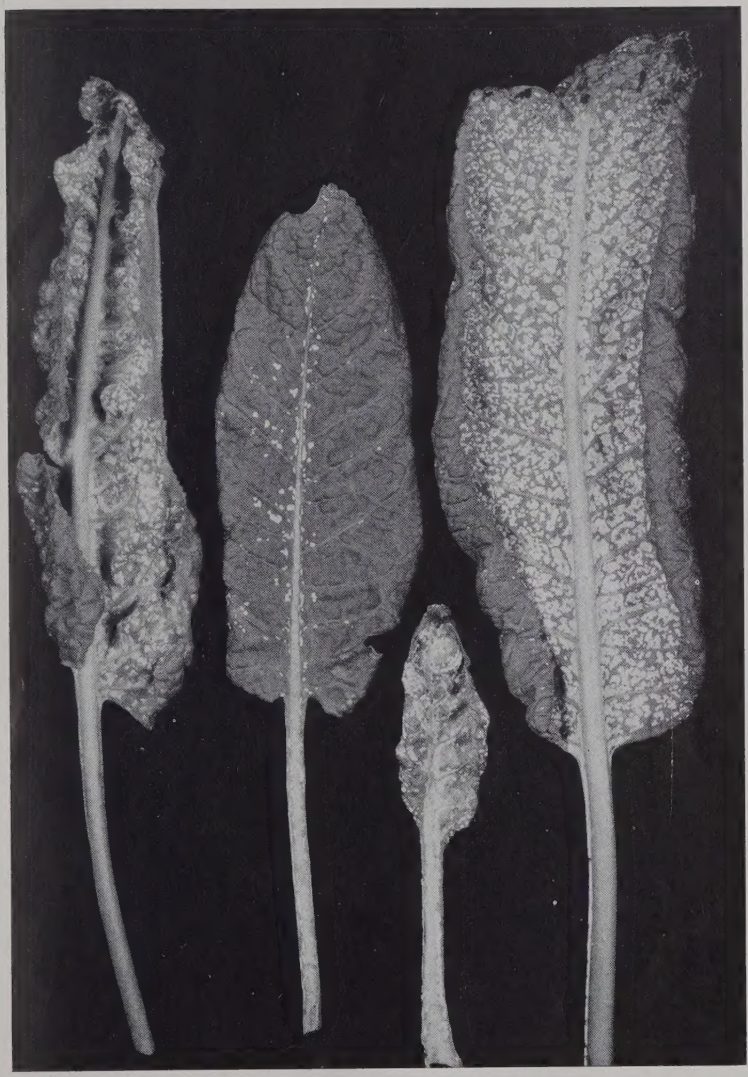
[3] *Armoracia rusticana* Gaertn., Mey. and Scherb.

If moderate temperatures prevail, individual sori sometimes coalesce to form larger pustules (Fig. 1) or successive concentric rings of sori may form (Fig. 3). Anthocyanin production is often induced by the fungus in the immediate area of the sorus and appears most prominently on the upper leaf surface (Fig. 4). Following dispersal of the conidia, the area of leaf occupied by the empty sorus usually dies. This happens most rapidly at temperatures above 28° C. The necrotic spots may then be confused with leaf spots caused by such fungi as *Alternaria brassicae* (Berk.) Sacc. and *Cercospora armorciae* Sacc.

Frequently the white-rust fungus becomes systemic in the crown region (Fig. 5) and occasionally in other areas of the root (Figs. 6, 7). When crown infection is present early in the spring, the fungus invades the shoot primordia, develops along with the growing shoot, and eventually produces sori on all aerial parts (Figs. 2, 8). Systemic infection may also spread from the crown into adjacent areas of the root or occasionally to the terminal end, and a second or even third extremely fasciculated cluster of infected roots or shoots may appear later in the season (Fig. 9).

Typical sori or pustules on underside of leaf. Note the variable size and shape of the sori. The large sori have resulted from the coalescence of several individual sori. (Fig. 1)



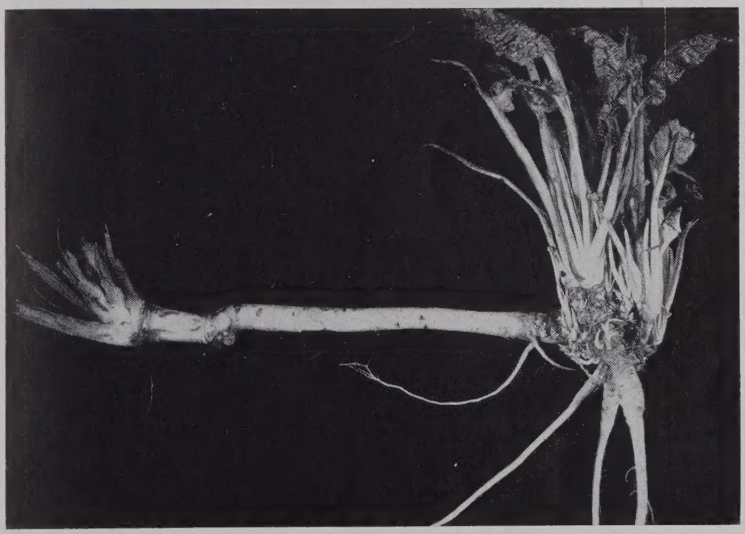
Systemically infected leaves. Note the dense formation of sori on the intercostal areas and petioles and the inward curling of the leaf. (Fig. 2)

Rings of secondary sori formed by the fungus as it spread out from the initial point of infection in the center of each ring. (Fig. 3)

The dark purple blotches on the upper surface of the leaf have been caused by the local stimulation of anthocyanin by the fungus. (Fig. 4)

Systemic infection of small leaves in the fall. The primary root shows a secondary invasion by bacteria which have spread rapidly down the root. (Fig. 5)

(*Below*) Systemic infection located only on the terminal end of the main root. Note the swelling of the secondary roots. (Fig. 6)

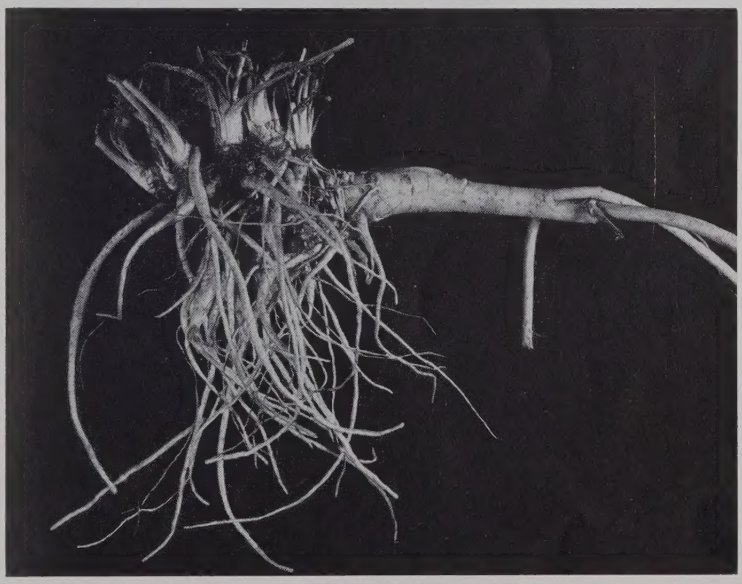


Two systemic infections over 3 inches apart, one located in the crown, the other in the middle of the main root. Serial sections of the root taken midway between the two infection foci revealed no trace of either mycelia or haustoria, indicating that the infections had arisen separately. (Fig. 7)

A single systemically infected shoot, appearing almost white, which arose during the spring from the crown area of a main root in the field. Note the extremely dense formation of sori. A single such leaf probably produces a million conidia under favorable conditions. (Fig. 8)

Systemically infected plants are easily recognized by the extreme density and almost simultaneous appearance of the sori on the underside of the leaves and on the extreme outer edge of the petioles (Fig. 2). Such leaves show an inward curling of the outer edges and are usually smaller than normal. In warm, dry weather, systemically infected leaves and petioles die quickly following conidia dispersal; in cool weather they may survive for 3 to 5 weeks.

The fungus, which does not readily advance up or down the petiole, is usually limited to areas about 2.5 cm. long and occasionally from 5 to 8 cm. long. Infected petiole and root tissue may be darkened either by mycelia (Fig. 10) or, less frequently, by the internal formation of oospores (Figs. 11, 12). Locally infected petioles frequently exhibit hypertrophy and hyperplasia (Fig. 13), though these symptoms are rare on systemically infected petioles or seed stalks (Fig. 14). Hypertrophy and hyperplasia are common in both locally and systemically infected roots (Figs. 10, 15). Sometimes they also occur in the veins of the leaf blade, but rarely in the intercostal leaf areas. Eventually, the pressure exerted by the continued internal cell division and by the formation of large, thin-walled, meristematic cells ruptures the epi-



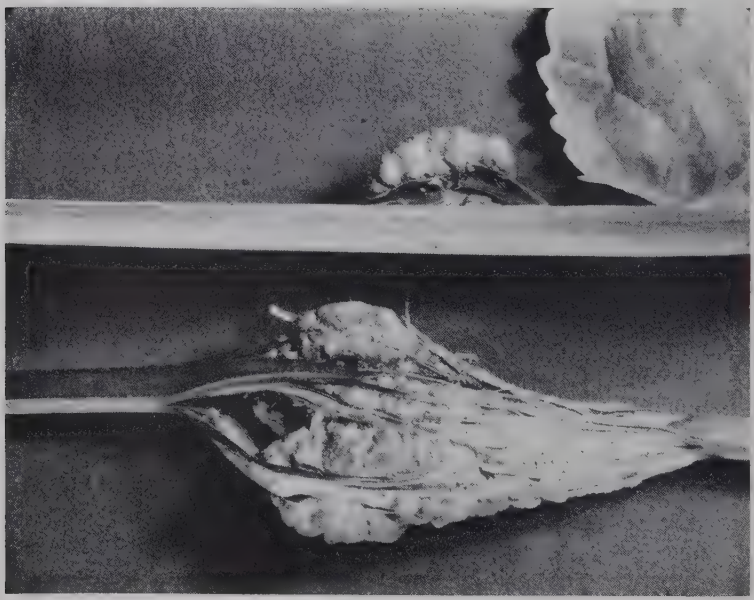
Extreme rosette of shoots and fasciculation of secondary roots. (Fig. 9)

dermis of the petiole or root. Such breaks in the epidermis of the root provide ready ingress for various rot-producing organisms, particularly bacteria (Fig. 16). Under moist conditions, these secondary organisms appear to contribute greatly to the destruction of the affected root.

Set roots affected by white rust. Note the swelling, discoloration, and cracking of the roots caused by mycelia. (Fig. 10)

Systemically infected crown showing root tissue darkly discolored by the internal formation of oospores. (Fig. 11)

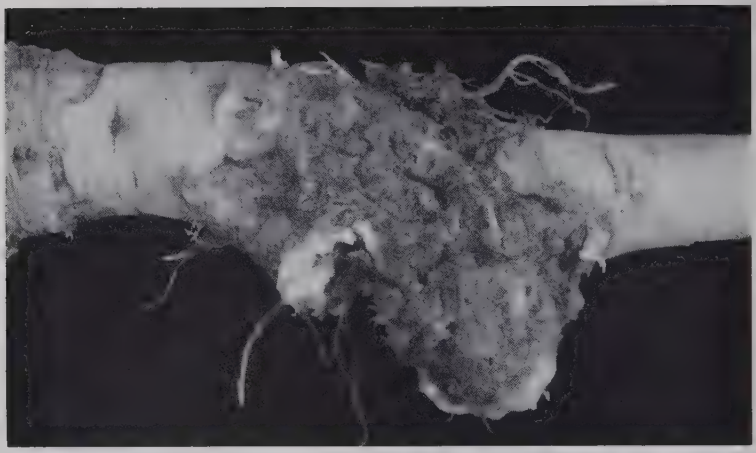
Two types of petiole discoloration. The lesions in the 2 petioles at the top extend to the surface and are believed to be symptoms of mosaic incited by turnip virus 1. The discoloration of the 2 petioles at the bottom does not extend to the surface and is due to the internal formation of oospores. (Fig. 12)



Localized petiole infection showing extensive swelling due to hypertrophy and hyperplasia. (Fig. 13)

Systemically infected seed stalks, all parts of which bear sori. (Fig. 14)

Infected primary root showing hypertrophy and hyperplasia. The swelling is also beginning to appear on the secondary roots. (Fig. 15)



Same root as in Fig. 15 with secondary roots removed. Note the drops of bacterial ooze and the wet rot caused by the bacteria. (Fig. 16)

LIFE CYCLE OF THE PATHOGEN

The intercellular hyphae of *Albugo candida* branch frequently and irregularly throughout the suscept tissue and form knoblike haustoria in the cells. Following a period of development in the leaf, the hyphae mass together in the large intercellular spaces of the spongy parenchyma beneath the epidermis. From this mass of hyphae arise sympodially branching hyphae which in turn produce club-shaped conidiophores. The conidiophores form great numbers of basipetal, catenulate, multinucleate conidia that eventually rupture the leaf epidermis. The conidia thus freed may be distributed by air currents. If they settle in a favorable environment of free moisture and suitable temperature, the conidia absorb water, swell, and divide internally into 5 to 8 uninucleate, biflagellate, reniform zoospores (swarmspores). The zoospores escape by dissolution of a specialized area, called the papilla, in the wall of the conidia. They swim for a period, with the length of time depending primarily on the temperature, then form a cell wall, lose their cilia, and germinate by means of a germ tube. Only rarely have conidia been observed to undergo direct germination by producing germ tubes instead of zoospores. Entrance into the plant is accomplished in most instances by the growth of the zoospore germ tube through the stoma. Thus the disease is reinitiated with many such secondary cycles occurring during the growing season.

A. candida also has a sexual stage which results in a thick-walled oospore, but this spore is rarely found in commercial horseradish fields and infection by oospores has not been accomplished in the greenhouse. The mature oospore has a diameter of 40 to 55μ, is globose, and possesses a dark, yellowish brown wall covered with low blunt ridges.

THE HORSERADISH PLANT

Varietal susceptibility

The so-called "common" horseradish, the only variety grown extensively in the United States, is susceptible to white rust. The Bohemian variety, also known as the Maliner Kren or Bayersdorf strain, possesses high resistance (8)[1] but is not grown extensively because of its poor root quality. Weber (20) in 1949 reported obtaining a very low percentage of viable seeds from a cross between certain clones of the common and of the Bohemian variety. Hougas *et al.* (8) tested 217 of these hybrid seedlings in the greenhouse for resistance to white rust. Some of these seedlings showed a resistance comparable to that manifested by certain clones of the Bohemian variety. The progenies of some of these crosses have undergone further evaluation for quality, adaptability, and disease reaction at the agricultural experiment stations of Wisconsin and Illinois.

Cultural practices

The horseradish, a biennial plant, is propagated by means of roots since the common variety is male sterile (20). Therefore, growers market the large primary root (Fig. 17) and save the secondary roots for planting the following year. These secondary roots, which are usually taken from the end of the primary root opposite the crown, are called "sets" or "whips." Long straight sets are in demand because when the sets are planted, they do not grow in length, only in girth. They may be removed from the primary root at harvest or at some later date. During the winter the sets are buried in shallow pits or held in cold storage. The marketable primary roots not sold immediately after harvest are stored temporarily in ground cellars.

The sets are planted in April or May in furrows about 3 feet apart. They are spaced about 1½ to 2 feet apart, from head end to head end, and at a slight angle with the soil line. Following planting, the set develops lateral and terminal roots. Growers remove the lateral roots

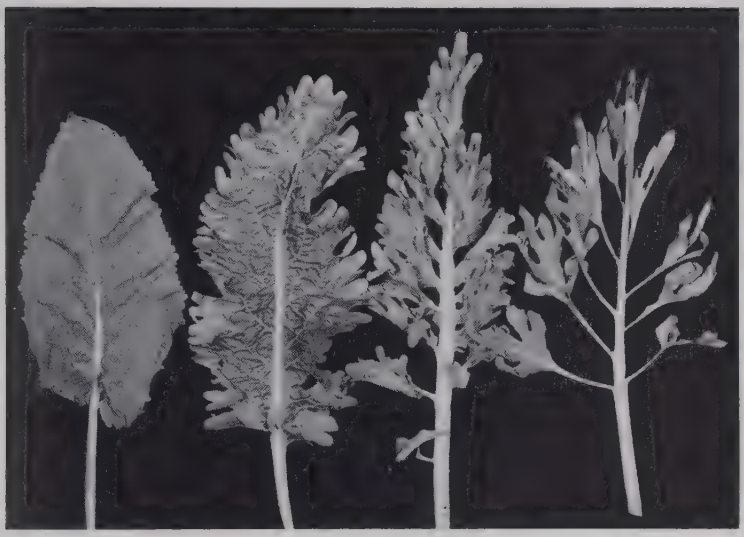
[1] Figures in parentheses refer to the bibliography on pages 55 and 56.

A normal horseradish root. The large primary root is sold; the terminal roots are used for next year's planting. (Fig. 17)

once or twice during the growing season by means of a special tool or break them off by simply lifting the plants partly out of the soil. This practice, called "lifting," results in a higher percentage of large primary roots. The roots are usually harvested during late October or November or left in the field over winter, depending on the market price.

Leaf types

Two types of leaves (Fig. 18) are produced by the horseradish plant during the growing season: (a) a simple, expanded leaf with undulate margins and (b) a deeply incised leaf somewhat resembling that of a fern. The simple type is formed from spring until midsummer. In August, leaves of the incised type begin to appear so that by late October most plants possess such leaves. When plants are allowed to overwinter in the field, they ordinarily produce incised leaves in the spring. Most plants will then produce seed stalks, usually sometime in May or June. The change in leaf type is, therefore, believed to be associated with a change from the vegetative to the reproductive stage. The factors responsible for this change are not known, but photoperiodism is probably involved. The stimulus ap-



Variation in shape of leaves of the common variety of horseradish. The simple broad leaf on the left prevails from planting until September. During late summer, the "cut-leaf" or "fern-leaf" type begins to appear and is dominant during the fall. (Fig. 18)

parently originates in the crown since portions of the primary root cut at a distance from the crown or secondary roots removed from plants bearing incised leaves usually will not maintain this character when replanted.[1]

METHODS AND MATERIALS

The common variety of horseradish was used in these investigations except for one experiment which tested for the presence of oospores in a resistant variety. The set roots were kept in cold storage until needed, then cut into 3- to 4-inch sections and each section planted in a 6-inch pot containing autoclaved soil. Plants with simple, expanded leaf blades were used in these studies unless otherwise specified.

Conidia of *Albugo candida* collected from horseradish grown at Urbana were used throughout these studies, since mass collections of conidia from the East St. Louis area, from Cook county, and from

[1] This relationship was first pointed out to the writers by Drs. R. W. Hougas and G. H. Rieman of the Wisconsin Agricultural Experiment Station.

Urbana caused identical disease reactions on the common variety of horseradish and, though less severely, on the Bohemian.

In the inoculation and disease development experiments, two inoculation procedures were employed. In the first, conidia were collected directly into distilled water maintained at the desired temperature and the resulting suspension atomized onto the lower leaf surfaces of the horseradish plants. The plants were then placed in an incubation chamber maintained at 100 percent relative humidity. During the summer, inoculations were made at night since day temperatures were too high for zoospore emergence and germination. The plants were usually left in the incubation chamber for 12 to 24 hours.

In the second method of inoculation, glass moist chambers 80 mm. x 240 mm. were filled with distilled water, placed in constant-temperature chambers, and maintained at 10°, 15°, 20°, 25°, 28°, or 30° C. for 24 hours before the experiment was initiated. The plants were also placed in the temperature chambers for 24 hours to allow them to adjust to the desired temperature. After 24 hours, a concentrated suspension of conidia (200,000 per cc.) was added to the distilled water in each of the moist chambers, and the leaves of the plants were immersed for 12 to 24 hours in the inoculum by inverting potted plants, supported by a wooden platform, over the suspension. The plants were removed from the suspension of conidia after different intervals, and the leaves dried by means of an electric fan, usually in 15 minutes or less. Very severe infection usually resulted from this method of inoculation which was particularly useful for obtaining an abundance of conidia of approximately the same age.

In all germination experiments, except in the experiments testing the effect of various temperatures on the rate of germination, conidia were collected in precooled, distilled water held at 15° C. They were removed from infected plants by means of the simple type of lung aspirator described by Raabe (15). The concentration of conidia was adjusted, usually from 100,000 to 250,000 conidia per cc., by means of a Levy hemocytometer. The suspension was shaken well and then dispersed by means of a pipette into 6 cm. petri plates.

All studies of the germination of conidia included counts both of zoospore release, as determined by the number of empty conidia, and of zoospore germination since not all the zoospores which were released produced germ tubes. The figures for zoospore release and zoospore germination are given separately in the tables and graphs that follow. The percentage of conidia releasing zoospores was determined by counting microscope fields at random until 100 conidia had been counted. Zoospore germination was considered to

have taken place when the germ tube attained a length equal to the diameter of the zoospore.

The glassware and distilled water used in the constant-temperature experiments were placed at the desired temperature at least 12 hours before the beginning of an experiment in order to allow them to adjust to the proper temperature. In preliminary studies deep-well glass slides were used, but germination was usually poor. Therefore, germination of conidia in petri plates (6 cm.) and in Erlenmeyer flasks (50 cc.) was compared with germination in the deep-well slides. Since higher germination was secured in the petri plates and Erlenmeyer flasks, sometimes by as much as 50 percent, they were used in subsequent germination studies, except for those involving relative humidity, for which microscope slides were found satisfactory. Aliquots were taken from the flasks at the desired intervals, and a drop of 0.01 percent osmic acid was added to the sample to stop germination.

The constant-temperature growth chambers were set at 10°, 15°, 20°, 25°, 28°, and 30° C. The 20° C. chamber varied $\pm$ 0.5° C.; all others varied $\pm$ 1.0° C. Relative humidity could not be controlled in the temperature chambers but was controlled in the moist chambers, when necessary, by means of salts or sulfuric acid.

GERMINATION OF CONIDIA

Direct germination is that which occurs when the conidia themselves produce germ tubes. Indirect germination occurs when the conidia release zoospores that produce germ tubes. Experiments performed under a wide variety of conditions showed that germination of *Albugo candida* is predominately indirect. Direct germination was rare and produced very short germ tubes, only 10 to 25μ long. The term "germination of conidia" in this paper refers both to zoospore release and to subsequent zoospore germination. The term "zoospore germination" refers to the production of germ tubes by zoospores.

Preliminary germination trials revealed that conidia collected from the field or greenhouse varied greatly in germination. Some collections germinated as high as 90 percent while others appeared to be completely dead. Frequently as high as 50 percent of the conidia released zoospores but none germinated. Imperfect release of zoospores as evidenced by 1 or 2 zoospores remaining within the conidium was also observed. Immature conidia or conidia that had become senescent or had lost their viability from other causes were occasionally seen to discharge their protoplasmic contents in a single undifferentiated mass. Usually this mass moved about vigorously in the water for a time,

lost its motility, and finally ceased all visible activity. Total disintegration of the protoplasmic mass was also noted. In extremely rare instances a very short germ tube or several germ tubes, usually less than 10μ long, were seen growing from such masses.

Type of water

Experiments were conducted to test the relative germination of conidia in rain water, tap water, distilled water, double-distilled water, and resin-exchange-treated water. Except for tap water, zoospore release and germination were nearly equal in all media. Distilled water was, therefore, used for germination and inoculation experiments. Free water was found to be necessary for germination. In seven trials, conidia exposed to a relative humidity of 100 percent and a temperature of 20° C. germinated only when moisture was allowed to condense on the glass slides.

Concentration of conidia

To determine if concentration was a factor in the germination of conidia, concentrations of 1,000, 10,000, 100,000, 250,000 and 500,000 conidia per cc. were prepared, and the conidia germinated in petri plates and on microscope slides at 15° C. for 24 hours. No significant difference in germination was observed at any of the concentrations. However, germination counts at concentrations of 250,000 conidia per cc. and above were very difficult to make because of the opaqueness imparted to the water by the conidia.

Light

To determine whether the presence or absence of light affected the germination of conidia, two sets of petri plates containing suspensions of conidia were prepared. One set was placed in a container made of aluminum foil and completely covered with two layers of black velvet cloth; the second set was left exposed to light. Each set of plates was then placed in temperature chambers adjusted to 10°, 15°, 20° or 25° C. Artificial illumination (250 foot-candles) was supplied from the outside of the temperature chambers. In three trials, each replicated three times, no consistent difference in germination was noted at any temperature between the conidia which received illumination and those which did not. Raabe and Pound (16) reported similar results with conidia of *A. occidentalis* and Melhus (10) with conidia of *A. candida*. No attempt was made in the present investigation to evaluate the effect of light quality, duration, or intensity upon the germination of conidia.

pH

In order to determine the relation of pH to germination, conidia at concentrations of 100,000 conidia per cc. were allowed to germinate at 15° C. for 24 hours in distilled water previously adjusted to various pH levels by the addition of .01 N hydrochloric acid or .01 N sodium hydroxide. Conidia germinated over the range of pH 3.5 to 9.5. Germination was adversely affected near the extremes but was good between 4.5 and 9.0, with the optimum at pH 6.5. After 24 hours the pH had shifted toward neutral. Hence, although pH 3.5 caused considerable zoospore disintegration, it was evident that pH exerts relatively little effect on either zoospore emergence or zoospore germ tube formation. The extreme pH values probably are never a limiting factor in nature. Raabe and Pound (16) obtained very similar results with conidia of *A. occidentalis*.

Age of sori

The relation of the age of the sori[1] of *Albugo candida*[2] to conidial germination has been investigated by Eberhardt (4), Melhus (10), and Napper (12). Eberhardt reported that age was the most important factor in germination, whereas Melhus believed that age was not a factor. Napper also concluded that within wide limits age was not a controlling factor.

Seasonal variations in the germination of conidia of different ages. The present study began with attempts to germinate conidia from sori of different ages on plants grown outdoors. As these experiments were repeated throughout the different seasons, it became apparent that conidia did not germinate as well during hot weather as during the rest of the year. Both the age at which the greatest percentage of germination was achieved and the percentage attained varied.

In the preliminary tests in the present investigation, conidia collected from mature sori during the months of November through May from field- or greenhouse-grown plants and placed in distilled water at 15° C. gave fair (45 percent) to good (79 percent) germination. Very young conidia from flat sori with intact epidermis often germinated very poorly. In general, germination appeared to increase in proportion to the age of the sori from which the conidia were collected. Following the advent of warm weather in June, conidia collected from field or greenhouse plants tended to germinate poorly at all stages of soral

[1] Since the conidia are produced in chains, conidia taken from any sorus will vary somewhat in age.

[2] *Cystopus candidus* Lév. in older literature.

development. Germination was poorest for conidia from sori just beginning to open. Greenhouse temperatures from July to September sometimes attained 43° C. and frequently remained at 35° C. for several hours daily. Napper (12) also obtained differences in germination of conidia of *A. candida* collected during different seasons, but she obtained better germination with conidia collected following warm, dry days than following cool, wet ones.

Temperature and sori formation. A more detailed experiment was started in mid-July, 1952, to determine the effect of temperature during sori formation on the subsequent germination of conidia. Horseradish plants were inoculated and left in the greenhouse until chlorotic areas appeared on the leaves. The plants were then placed in temperature chambers maintained at 10°, 15°, 20°, 25°, and 30° C. As soon as sori began to appear, the conidia were collected daily from plants in each of the constant-temperature chambers, sown in water, and germinated at 15° C. for 24 hours. The collection and germination of conidia were discontinued when rupture of the soral epidermis became general. The average of six experiments is presented in Table 1. A portion of the results is also shown graphically in Figs. 19 and 20.

The data show that the age of the sori from which the conidia are taken is definitely a factor in their germination. The percentage of conidia that released zoospores and the percentage of zoospore germination gradually increased as the age of the sori from which the conidia were collected increased. Conidia produced at a constant temperature germinated in much higher numbers and more consistently than conidia collected from plants grown in the field or greenhouse.

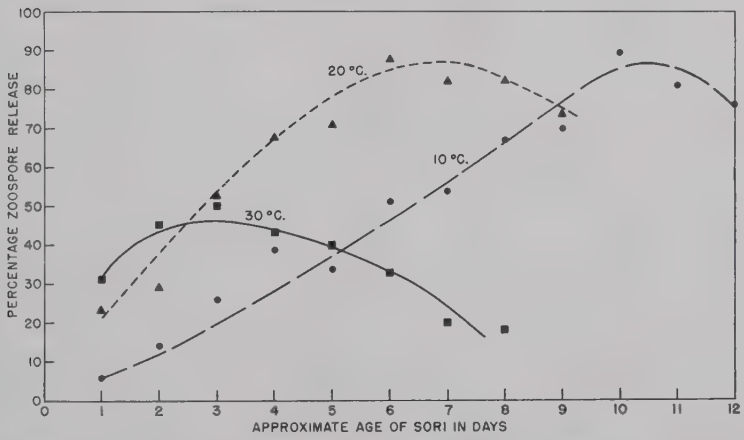
Table 1. — Effect of Temperature During Sori Formation on the Subsequent Germination of Conidia

Temperature	Stage of germination of conidia	Age of sori in days											
		1	2	3	4	5	6	7	8	9	10	11	12
		Percent germination											
10° C.	zoospore emergence	6	14	26	39	34	51	54	67	70	89	81	76
	zoospore germination	0	3	25	36	42	63	61	79	88	86	77	71
15° C.	zoospore emergence	15	27	48	55	68	71	86	78	79	..	..	..
	zoospore germination	4	13	61	65	64	55	89	81	73	..	..	..
20° C.	zoospore emergence	23	29	52	68	71	88	82	82	79	..	..	..
	zoospore germination	34	40	68	71	72	79	78	71	63	..	..	..
25° C.	zoospore emergence	36	41	64	69	83	77	66	61	..	..	..	..
	zoospore germination	27	54	51	61	84	60	50	63	..	..	..	..
30° C.	zoospore emergence	31	45	50	43	40	33	20	18	16	7	..	..
	zoospore germination	15	33	44	44	43	49	19	14	11	3	..	..

As expected, temperature influenced the rate at which conidia were able to germinate well. The time required for development in the sori shortened as the temperature increased; for example, conidia formed at a temperature of 10° C. required 9 to 11 days to attain their maximum range of germination, whereas conidia formed at 30° C. required only from 2 to 6 days. However, conidia formed at 30° C. did not release as high a percentage of zoospores as conidia produced at lower temperatures, nor did those zoospores germinate as well as the other zoospores. The results also indicate that at high temperatures a loss of viability may begin before the sori rupture.

To determine the effect of low temperatures on the formation of conidia within the sori, immature conidia were placed in distilled water at temperatures ranging from 3° to 5° C. These conidia had been collected from very flat sori between 2 and 3 days old that had been produced at a temperature of 10° C. At 3° to 5° C. zoospore emergence from mature conidia normally occurs in 8 to 12 hours. But when these immature, detached conidia were germinated at 3° to 5° C., some of them released zoospores within 24 to 48 hours, others after 7 to 10 days, while still others did not release zoospores at all.

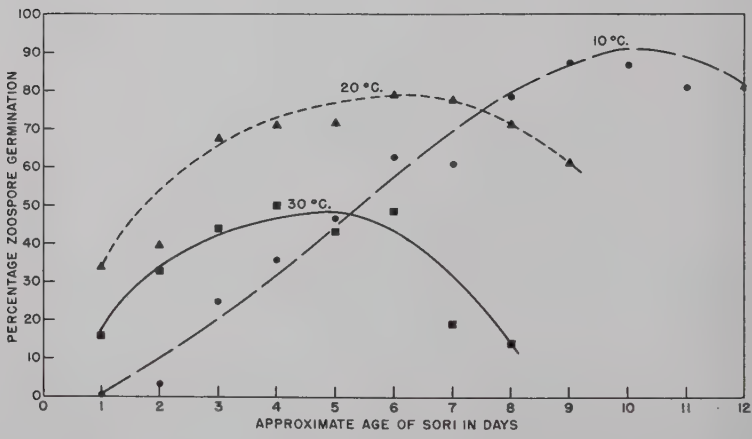
A second experiment showed that the immaturity of the conidia when detached and not the extreme cold was the cause of their failure to germinate. Plants which had been placed at 10° C. when chlorotic



Effect of temperature during sori formation on the germination of conidia of *A. candida* as measured by the percentage of conidia releasing zoospores. The conidia were germinated at 15° C. for 24 hours. (Fig. 19)

areas appeared were transferred to 1° to 3° C. as soon as flat sori were observed. The sori were allowed to develop at this temperature, and samples of conidia were taken at intervals starting 5 days later and continuing until the sori were 21 days old. At first, only a few conidia released zoospores and usually these zoospores did not form germ tubes or only very short ones. After several days, however, a greater percentage of conidia released zoospores; a greater percentage of the zoospores germinated; and the time required for conidia to start to germinate after they were released was shortened. The reason some conidia did not need to remain in the sori as long as others after being subjected to low temperature probably is that they were already closer to maturity. The probable reason why some of the immature, detached conidia described in the preceding paragraph failed to germinate is that conidia cannot continue to develop unless they have attained a certain degree of development while still attached to the parent hyphae.

It is apparent that age is an important factor in the germination of conidia of *A. candida.* This is in accordance with the earlier work of Eberhardt (4, 5) with *A. candida* and of Raabe and Pound (16) with *A. occidentalis.* In addition, the higher the temperature, within the permissible range, the less time conidia needed to remain in the sori in order to attain maximum germination. At the highest temperature, they appeared to have lost their viability before the sori ruptured.



Effect of temperature during sori formation on the germination of conidia of *A. candida* as measured by the percentage of zoospore germination. The conidia were germinated at 15° C. for 24 hours. (Fig. 20)

Desiccation

Though it has been demonstrated that conidia of *Albugo candida* cannot germinate except in free water, there remain the problems of the effect on the germination of conidia of the loss of water by the leaf while the conidia are still in the sori and of the effect on germination of exposing the conidia to a low relative humidity after they have been freed but before water is available for germination.

Leaf desiccation and wilting. Napper (12) studied the effect of leaf desiccation on conidia of *A. candida,* and Raabe and Pound (16) studied the effects both of drying leaves and of wilting whole spinach plants on the germination of *A. occidentalis.* Napper concluded, on the basis of her data, that conidia of *A. candida* will not germinate unless their water content has been reduced by approximately 30 percent. Similarly, Raabe and Pound showed that germination of conidia from desiccated plants was better than from normal turgid ones. In both instances the investigators were measuring the percentage of water lost by the suscept rather than by the conidia, which Napper realized might not be the same because of a difference in osmotic gradient.

In the present investigation, the methods used by Napper and by Raabe and Pound were repeated. To test the effect of leaf desiccation, leaves from plants grown in the greenhouse or grown at a constant temperature of 20° C., were weighed and then air-dried on a laboratory bench. Samples of conidia were taken at different intervals to determine what percentages released zoospores, and the leaves were weighed on each occasion to determine the amount of the moisture loss. Conidia were taken from sori in three developmental stages: (a) flat, grayish white sori (1½ to 2 days old) with intact epidermis, (b) erumpent, creamy white sori (5 to 6 days old) with intact epidermis, and (c) sori with the epidermis ready to break (7 to 9 days old). Conidia from non-detached leaves were used as a check. Although moisture loss was not necessary for zoospore release by conidia of *A. candida* in these tests, conidia sometimes released zoospores slightly better when the detached leaves they were on lost 30 percent or more moisture (Table 2).

In another experiment, germination tests were run on 6-day-old conidia collected from wilted greenhouse plants. Wilting of the entire plant was decisively unfavorable for conidia of *A. candida.* The conidia on wilted leaves had a low of 2.5 percent zoospore release, a high of 54.5, and an average of 18.22. Conidia from leaves of turgid control plants had a low of 23.5 percent, a high of 79.5, and an average of 52.54.

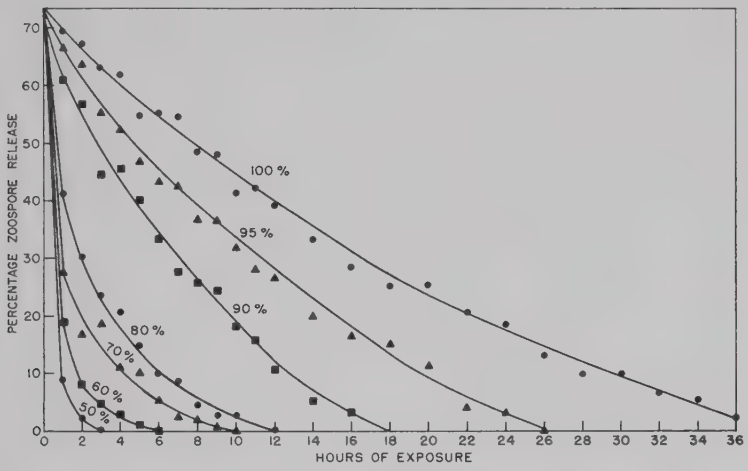
Table 2. — Effect of Desiccation of Leaves Bearing Sori Upon Zoospore Release by Conidia of *Albugo candida* Obtained From Plants Grown in the Greenhouse (Average of 5 trials) or Grown at the Constant Temperature of 20° C. (Average of 3 trials)

1- to 2-day-old sori			5- to 6-day-old sori			8- to 9-day-old sori		
Percent water loss	Percent zoospore release	Check	Percent water loss	Percent zoospore release	Check	Percent water loss	Percent zoospore release	Check
				Greenhouse				
13	9	10	18	66	69	15	51	40
30	11	10	29	67	56	23	50	39
36	6	8	35	44	69	30	41	48
49	4	6	52	16	55	38	46	49
						50	23	37
				Constant temperature				
12	15	10	17	75	68	10	54	50
26	15	19	25	69	63	24	47	53
33	18	6	39	40	77	37	35	42
42	4	12	48	24	81	49	6	44

Napper's claim that conidia must lose water to release zoospores could not be confirmed, although there is some evidence that zoospore release from conidia from sori six days old or older may at times be improved by leaf desiccation.

Relative humidity. The effect of desiccating conidia was studied in a third experiment in which detached conidia were exposed to different relative humidities for intervals of 1 to 36 hours and then placed in distilled water at 15° C. to germinate. This experiment did not attempt to measure directly the water lost by the conidia, but it did measure (1) the percentage of conidia that released zoospores after a given interval of exposure to a given degree of relative humidity, and (2) the duration for which conidia could be exposed to a given degree of relative humidity and still germinate.

Conidia from sori six days old were dusted onto clean, dry glass slides, placed in glass moist chambers at 20° C., and exposed to relative humidities of 100, 95, 90, 80, 70, 60, and 50 percent. Each relative humidity was maintained by various concentrations of salts or sulfuric acid. The slides were removed at 1-hour intervals during a period of 12 hours and at 2-hour intervals thereafter for an additional 24 hours. Each treatment was replicated twice and the experiment carried out three times. Release of zoospores by the conidia decreased rapidly with exposure to decreasing relative humidities and with an increase in



Relation of relative humidity to zoospore release by conidia at 20° C. Each point on the curve is based on an average of 3 trials, replicated 2 times. The conidia were taken from 5- to 6-day-old sori produced at a constant temperature of 20° C. (Fig. 21)

exposure time (Fig. 21). In this experiment, the check conidia averaged 75 percent zoospore release.

The data show that relative humidities of 50 to 80 percent at 20° C. are unfavorable for retaining the viability of conidia. Zoospore release fell off very rapidly, and at the end of 6 hours only 10 percent of the conidia exposed to a relative humidity of 80 percent were able to release zoospores. Conidia exposed to lower relative humidities were correspondingly affected. At relative humidities of 90 to 100 percent, zoospore release fell off less rapidly, but at the end of 24 hours of exposure, the percentage of conidia able to release zoospores was down to 3 percent for those exposed to 95 percent relative humidity and to 20 percent for those exposed to 100 percent relative humidity. The germ tubes produced by zoospores released after 24 hours at 100 percent relative humidity were very short. The conidia in this experiment were not tested for pathogenicity.

It is apparent from the tests on relative humidity that regardless of whether a moderate desiccation of plants favors germination of conidia, a direct exposure of conidia to desiccation soon stops or greatly reduces their ability to release zoospores, even at high relative humidi-

ties. It is also apparent that despite leaf desiccation up to a moderate degree of wilting, the attached conidia are able to retain enough moisture to permit zoospore release.[1]

Cardinal temperatures

The cardinal (i.e. minimum, optimum, and maximum) temperatures for conidial germination were determined at the end of 24 and 48 hours. These temperatures apply to indirect conidial germination as defined on page 19. Both young and old conidia were used in order to test conidia having optimum and reduced viability. Young conidia were collected from sori 6 to 7 days old on plants that had been placed in the 20° C. constant-temperature chamber as soon as the chlorotic areas appeared. Old conidia were taken from greenhouse-grown plants bearing open sori that had turned a dirty-gray color and from which most of the conidia had fallen. The conidia were collected directly in distilled water previously adjusted to the appropriate temperature and were allowed to germinate at temperatures of 0°–2°, 5°–7°, 10°, 15°, 20°, 25°, 28°, and 30° C.

The data (Table 3) show that conidia from young sori germinated at higher and lower temperatures and with far greater intensity at all temperatures than did conidia from old sori. Zoospore release by conidia from young sori was favored by the temperatures of 10° to 20° C., and zoospore release by conidia from old sori by the temperatures of 10° and 15° C. Higher temperatures greatly reduced zoospore release. Germination by zoospores from conidia from young sori was favored by temperatures from 10° to 25° C., and germination by zoospores from conidia from old sori by temperatures from 10° to 20° C. Temperatures higher than these greatly reduced zoospore germination. At almost every temperature and for conidia from both young and old sori, the percentage of zoospores that produced germ tubes was higher than the percentage of conidia which released zoospores.

In these experiments, the minimum, optimum, and maximum temperatures were 0°, 15°, and 28° C., respectively. Allowance should be made, however, for the variation in results obtained from different tests. Zoospore release and zoospore germination after 24 hours were nearly as good at 20° as at 15° C. in the rate of germination experiments. Also, the best results for germ tube growth were obtained at 20° C., and the same was true for the experiment determining what

[1] See also page 43 on the watering of plants and the length of conidial survival under refrigeration.

Table 3. — Effect of Temperature During Germination Upon the Release of Zoospores by Conidia and Upon Zoospore Germination (Average of 14 trials)

Temperature	Young conidia				Old conidia			
	Zoospore release		Zoospore germination		Zoospore release		Zoospore germination	
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
	(percent)							
0°-2° C.	6	16	12	19	0	0	0	0
5°-6° C.	21	38	39	63	2	4	14	18
10° C.	71	77	83	91	26	30	28	38
15° C.	79	85	87	92	39	36	35	37
20° C.	64	65	79	88	13	16	30	32
25° C.	24	31	64	67	4	6	2	9
28° C.	1	2	4	8	0	0	0	0
30° C.	0	0	0	0	0	0	0	0

temperature best favored conidial germination and entrance into the leaf. Therefore, although 15° C. was the optimum temperature for zoospore release and germination in all experiments, it may be better to speak of 15° to 20° C. as the optimum temperature range for germination. The cardinal temperatures given here are in fairly good agreement with those reported by Napper (12), DeBary (2, 3), and Melhus (10). Napper (12) reported a maximum temperature for germination of about 20° C., while DeBary and Melhus found this to be around 25° C.

Chilling

Exposure of the conidia of *A. occidentalis* to 12° C. (nearly optimum for germination) was reported by Raabe and Pound (16) to give a higher germination at other temperatures than was obtained from conidia not so preconditioned. Conidia were placed at 12° C. for 1½ hours and then held at 4°, 8°, 12°, 16°, 20°, and 24° C. Raabe and Pound stated that, "Not only did the chilled conidia germinate at higher temperatures, but germination was about the same at all temperatures."

In the present investigation, conidia produced at 20° C., the optimum for good germinability, were collected at 2 to 3 and at 5 to 6 days after the first appearance of sori. The conidia were placed at 10° C., and, at 30-minute intervals, aliquots of the suspension were removed and put in petri dishes at 15°, 20°, 25°, 28°, and 30° C. for 24 hours

each. This experiment, which was continued until maximum zoospore release had occurred, was repeated eight times.

If the conidia were transferred from 10° C. before zoospore release started, germination at each temperature was less than that in the checks at the same temperatures. If the conidia were transferred while releasing zoospores, an increase in germination was sometimes observed, regardless of the age of the sori from which the conidia were taken. But only when zoospore release was allowed to proceed to its apparent maximum at 10° C. before the zoospores were transferred to the higher temperatures, did germination usually increase. This is not surprising, since holding conidia at 10° C. permitted them to release zoospores at a temperature which, though it takes a long time, is conducive to a very high maximum release; and since raising the temperature to 15° or 20° C. after maximum zoospore release had occurred put the zoospores in the optimum range for zoospore germination.

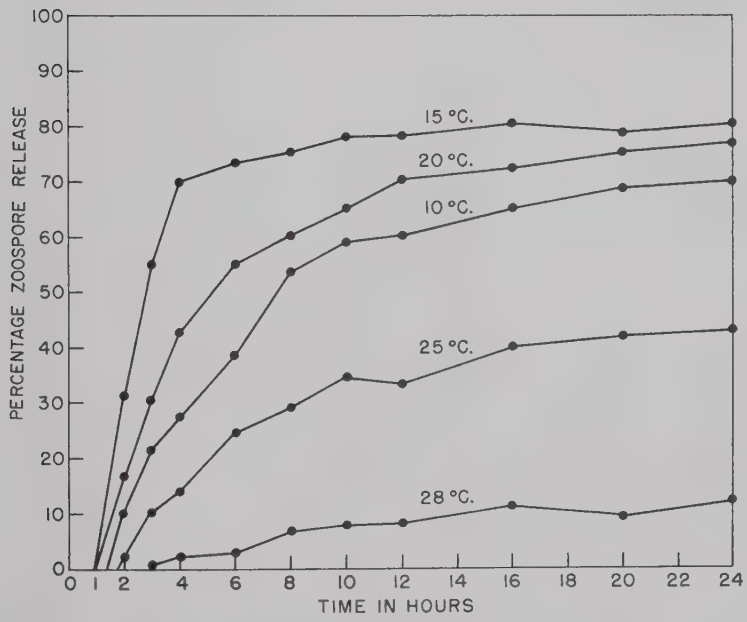
Temperature and rate of germination

Investigators who have studied the relationship between temperature and germination of conidia of *Albugo candida* have recorded their results after 24 or 48 hours; no attempt has been made to determine the effect of temperature upon the rate of germination. But if free moisture is present for only a few hours on the infection court, zoospore release by conidia and the subsequent germ tube production by zoospores must be accomplished quickly in order for the germ tubes to enter the suscept and initiate infection. Therefore, rapidity of germination over a short period is frequently critical in determining the intensity of infection.

Conidia of 5- to 6-day-old sori from leaves of infected plants grown at 20° C. were collected directly into distilled water previously adjusted to the appropriate temperature and were germinated at 10°, 15°, 20°, 25°, and 28° C. A drop of dilute osmic acid was added to the samples to stop germination at the end of the interval being tested. The effect of temperature upon the rate of zoospore emergence, duration of zoospore motility, rate of zoospore germination, and rate of germ tube elongation was determined by taking numerous readings at predetermined intervals over a period of 24 hours after sowing the conidia in water. The effect on germination of briefly exposing conidia to slightly higher temperatures than they can endure continuously was also tested.

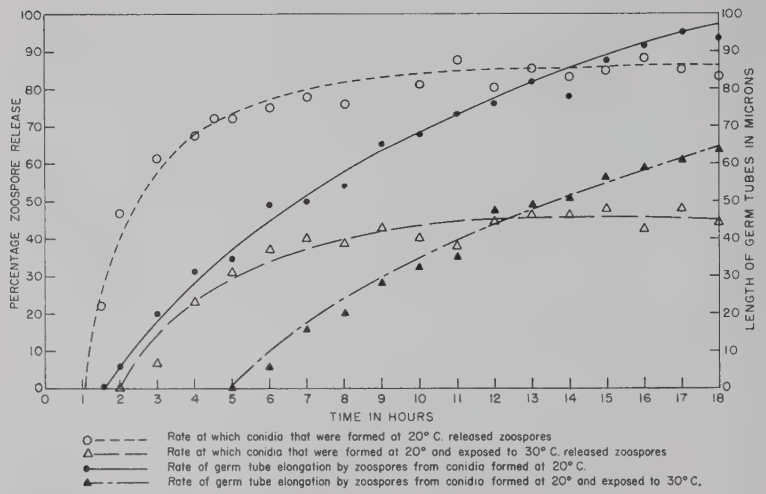
Zoospore release. Other factors permitting, the intensity of infection is largely determined by the rate at which zoospores emerge

from the conidia. At the end of three hours (Fig. 22), 55 percent of the conidia had released zoospores at 15° C., and 0.5 percent at 28° C. Zoospore emergence first occurred at 15° C. in slightly less than 1 hour. Temperatures between 10° and 20° C. were favorable for a high rate and percentage of zoospore emergence. (Old conidia have a narrower range of 10°-15° C. for efficient emergence.) The optimum temperature probably is close to 15° C. High temperatures of 25° C. and above caused a loss of viability; for example, conidia produced at 28° C. generally required from 1 to 4 hours longer to germinate than conidia produced between 10° and 20° C.

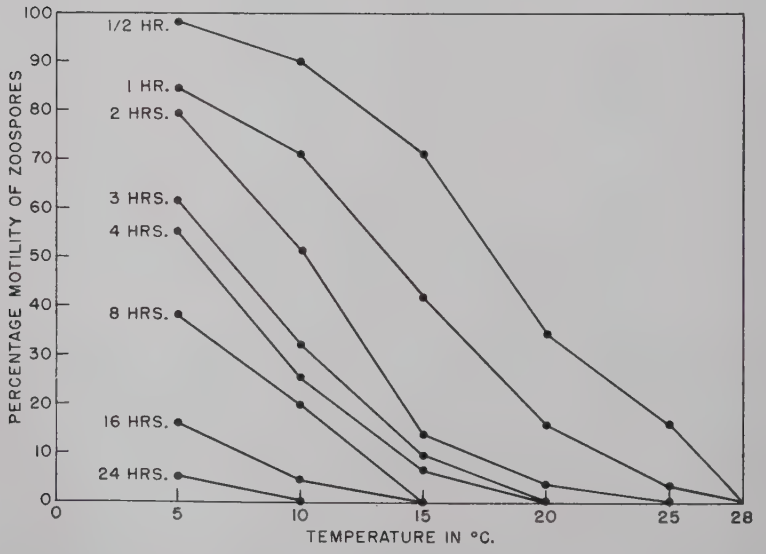


Effect of temperature during germination on the rate at which conidia release zoospores. Each point on the curve is based on an average of 4 trials, replicated 2 times. The conidia were taken from 5- to 6-day-old sori produced at 20° C. (Fig. 22)

High temperatures and subsequent conidial germination. To determine the effect of a brief high temperature exposure on the subsequent germination of conidia, plants with sori 5 to 6 days old and produced at 20° C. were placed in constant-temperature chambers adjusted to 30° C. for intervals up to 12 hours. Then the conidia were


PERCENTAGE ZOOSPORE RELEASE
LENGTH OF GERM TUBES IN MICRONS
TIME IN HOURS
Rate at which conidia that were formed at 20° C. released zoospores
Rate at which conidia that were formed at 20° and exposed to 30° C. released zoospores
Rate of germ tube elongation by zoospores from conidia formed at 20° C.
Rate of germ tube elongation by zoospores from conidia formed at 20° and exposed to 30° C.

Effect on the subsequent germination of conidia of exposing them to a high temperature while they were still in the sori. Each point on the curve is an average of 3 trials replicated 3 times. The conidia were germinated at 15° C. for 24 hours. (Fig. 23)

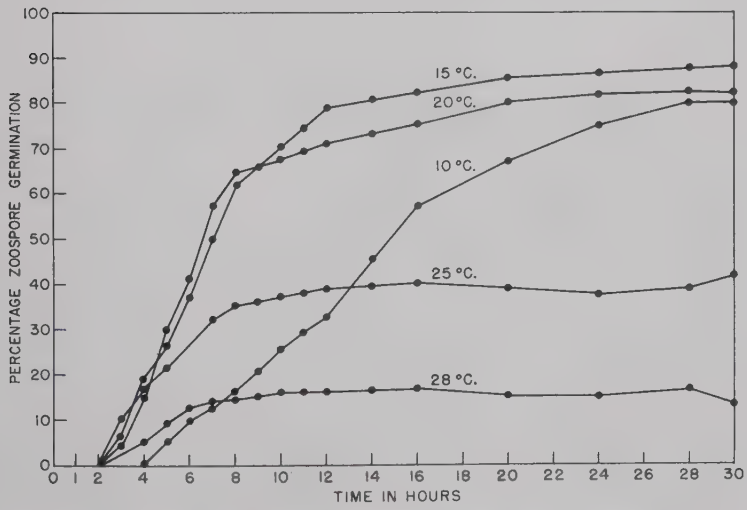


Effect of temperature during germination on the duration of zoospore motility. Each point on the curve is based on an average of 4 trials, replicated 2 times. The conidia were taken from 5- to 6-day-old sori produced at a constant temperature of 20° C. (Fig. 24)

collected and germinated at 15° C. for 24 hours. The effect of a brief high temperature exposure is shown graphically in Fig. 23. The conidia in this experiment were of identical ages; however, one set of plants had been exposed to a temperature of 30° C. for 12 hours while the other set was left at 20° C. In general, the time required for conidial germination increased and the rate and percentage of germination decreased with increasing exposure to 30° C.

Zoospore motility. The data (Fig. 24) on the relation of temperature to zoospore motility confirm the findings of previous investigators, namely, zoospores rapidly lose their motility at moderate to high temperatures (15° to 28° C.) and retain their motility at low temperatures (5° to 10° C.). At temperatures of 15° C. or above, less than 20 percent of the zoospores still have their motility after 2 hours, while at 10° C., 50 percent are active, and at 5° C., 80 percent are active at the end of two hours.

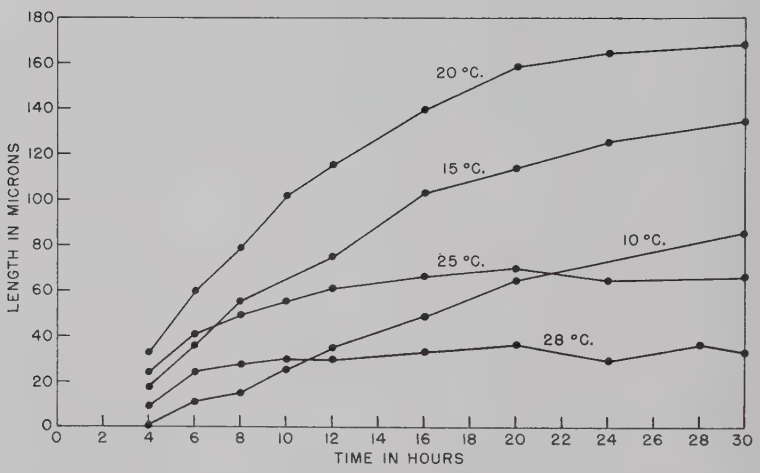
Zoospore germination. Temperature influenced the rate of zoospore germination (Fig. 25). Less than 2 percent of the zoospores had produced germ tubes 2 hours after conidia were placed in water



Relation of temperature during germination to the rate of zoospore germination. Each point on the curve is based on an average of 4 trials, replicated 2 times. The conidia were taken from 5- to 6-day-old sori produced at a constant temperature of 20° C. (Fig. 25)

at 15° C. or warmer, and none before 4 hours at 10° C. During the first 9 hours, zoospore germination was practically the same for 15° and 20° C. After this time, the percentage of germination was slightly better at 15° C.

Germ tube elongation. Temperature also affected the rate at which zoospore germ tubes elongated (Fig. 26). The rate of elongation was greatest at 20° C. At 6 hours, germ tubes grown at 20° C. were 60 microns long, and at 20 hours they reached 160 microns. The germ tubes which formed at 28° C. were usually very short and occasionally branched. At the end of 48 hours, germ tubes formed at 10° C. were usually as long as those formed at 15° or 20°. These results show that the best germ tube growth, as measured by rate of elongation, occurs at approximately 20° C.



Relation of temperature during zoospore germ tube production to the rate of elongation of the germ tubes. Each point on the curve is based on an average of 4 trials, replicated 2 times. The conidia were taken from 5- to 6-day-old sori produced at a constant temperature of 20° C. (Fig. 26)

INITIATION AND DEVELOPMENT OF THE DISEASE

The intensity of local infection on leaves depends on the supply of conidia, on the presence or absence of free water, and on the temperature during the germination of the conidia on the leaf. After

entrance into the leaves has occurred, the length of the incubation period and the degree of disease development depend primarily on temperature.

Intermittent versus continuous wetting of leaves

Various methods of inoculation and of maintaining a moist infection court were compared. The plants were inoculated (a) by dusting them with a mixture of conidia and talc, (b) by spraying them with a suspension of conidia, or (c) by immersing them in a suspension of conidia. Those which were dusted or sprayed were wetted, either intermittently or continuously, with an atomized spray of water for 24 hours. During intermittent atomization, the plants were wetted for 4 hours, then left to dry for 4 hours, and this alternation was continued for the 24-hour period. Separate glass chambers were used for the intermittent and continuous atomization. The immersed leaves were left in the suspension for 24 hours.

The degree of infection resulting from the different methods of inoculation was measured by the following visual intensity-of-infection index: trace = 1-2 sori per leaf; 1 = 3-50; 2 = 51-100; 3 = 101-250; 4 = 251-500; 5 = more than 500. The data (Table 4) show that heavy infection occurred only when the leaves were kept continuously

Table 4. — Incidence and Degree of Infection as a Result of Intermittent and Continuous Wetting of Leaves (24 hours at 16° to 20° C.)

Method of inoculation	Number of plants inoculated	Number of plants infected and infection index[a]					
		Intermittent atomization		Continuous atomization		Continuous immersion in suspension of conidia	
		Number	Index	Number	Index	Number	Index
Conidia-talc mixture[b] dusted on leaves	18	6	0-1	15	trace-1	..	..
Conidia suspension sprayed on leaves	18	10	1-2	18	3-5	..	..
Leaves immersed in suspension of conidia	18	..	..	..	..	18	4-5

[a] For basis for index of infection, see above.
[b] Conidia were scraped from sori and added to talc at a ratio of 1:5.

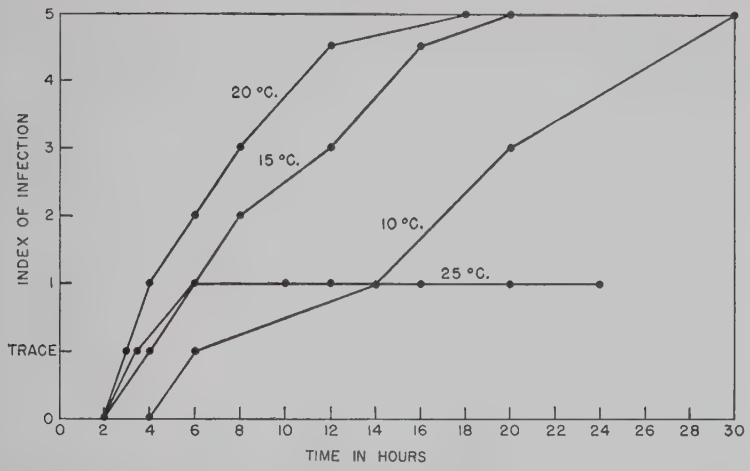
wet either by constant atomizing or by immersing the leaves in a suspension of conidia. These results are in agreement with the findings of Hougas *et al.* (8) and Raabe (15), i.e., that conidial germination and germ tube entrance require the presence of free water.

Temperature and germ tube entrance

The influence of temperature on the length of time required for germ tube entrance has not been investigated for any species of Albugo, though of course the time required for entrance might be expected to approximate the time necessary in distilled water for conidia to produce germ tubes by indirect germination. However, several workers have reported various minimum periods of exposure to inoculum required for infection. Napper (12) working with *Albugo candida* reported that the zoospore germ tubes could enter the suscept through the stomata and establish the first haustoria in the suscept cells within a "few hours" after inoculation. Hougas *et al.* (8) tested horseradish seedlings for resistance to *A. candida* and reported that a period of 4 hours in a saturated atmosphere was sufficient to obtain good infection. Raabe (15) reported that infection of spinach with *A. occidentalis* occurred in 2 hours in a saturated atmosphere although the spinach plants usually were left in the moist chamber for 48 hours.

In the present investigation plants were inoculated by immersing the leaves for varying periods of time in a suspension of conidia held at 10°, 15°, 20°, 25°, 28°, or 30° C. At the end of each period of inoculation, the leaves were dried with an electric fan, usually in fifteen minutes or less.

The visual intensity-of-infection scale described on page 35 was used in these tests. The amount of infection was usually determined 10 to 15 days after inoculation (Fig. 27). A total of 258 plants were used in this experiment which was repeated 5 times. A trace amount of infection (1-2 sori per leaf) resulted from 3 hours of immersion at 20° and 15° C., 4 hours at 25° C., and 6 hours at 10° C. The temperature of 20° C. was most favorable for infection, and 15° C. was only slightly less so since the maximum intensity of infection occurred at these temperatures after 18 and 20 hours of immersion, respectively. At 10° C. the time required for infection was greatly lengthened and maximum infection was obtained only after 30 hours. For the first 6 hours at 25° C., the intensity of infection almost equaled that occurring at 15° C. but then leveled off. The maximum temperature for infection was probably 28° C. since only zero-to-trace infection occurred at this temperature. The minimum time needed to achieve entrance is in fairly close agreement with the minimum times of



Relation of temperature during germination on the leaf to the time required for entrance. Each point on the curve is based on an average of 5 trials, replicated 2 times. See page 35 for the Intensity-of-Infection Index. (Fig. 27)

zoospore release, zoospore germination, and germ tube growth in distilled water. The complete process of conidial germination in distilled water, as represented by germ tubes capable of entrance, was fastest also at 20° C.

Temperature and the length of incubation

The effect of temperature on the length of time required for incubation[1] and for the production of sori was determined as follows: Plants were inoculated in the greenhouse and, after 12 hours in the moist chamber, were removed and incubated in constant-temperature chambers regulated at 10°, 15°, 20°, 25°, 28°, 30°, and 35° C.

The most favorable temperature for incubation with respect to time was between 25° and 28° C. (Table 5). However, temperatures between 15° and 25° C. resulted in the greatest number of sori per unit area of leaf.

Though the fungus cannot develop at all at a constant temperature of 35° C., another experiment showed that sori did develop at 35° C. if they were initially incubated at 20° C. for at least 4 days after inoculation. However, even after an initial incubation of 6 days at 20° C., development was poor. Likewise, the germination of conidia from

[1] The period between inoculation and the appearance of chlorotic areas.

Table 5. — Effect of Temperature During Incubation on the Number of Days After Inoculation Until the Appearance of Chlorotic Areas and of Sori, and on the Intensity of Infection

Temperature	Time in days for appearance of:		Index of infection
	Chlorotic areas	Sori	
10° C.	16-20	18-24	2-5
15° C.	7-9	9-12	3-5
20° C.	6-8	8-10	3-5
25° C.	5-8	7-9	3-4
28° C.	5-7	6-9	trace-2
30° C.	7-9	9-13	trace-1
35° C.	[a]	[a]	[a]

[a] No symptoms appeared at this temperature.

sori incubated at 30° C. improved as the plants were held at 20° C. for 1 to 6 days after inoculation.

The effect of day and night temperatures throughout the year on the length of the incubation period was determined by inoculating and incubating plants in the greenhouse. The minimum and maximum greenhouse temperatures during several experiments in July and August were 24° and 40° C., respectively, and the average was 29° C. Chlorotic blotches appeared on the leaves in about 11 days, and production of conidia, which was poor, began in 11 to 13 days. The index of infection varied from a trace to 3. The conidia taken from such plants usually germinated poorly (7 to 33 percent), and produced infection measuring only from a trace to 2. In addition, the fungus died soon after the rupture of the soral epidermis and did not form secondary rings. Plants incubated in the greenhouse during late fall, winter, and early spring developed chlorotic areas in 6 to 8 days and began to produce sori in 8 to 11 days. Conidia collected from such plants before the rupture of the soral epidermis occurred, or shortly thereafter, usually germinated well. In addition, the fungus frequently spread out from the primary sori to form secondary rings of sori.

Temperature and the production of sori, size of sori, and development of secondary sori

Raabe and Pound (16) studied the effect of temperature on the production of conidia and on disease development in the white-rust disease of spinach. They found that the disease developed more rapidly at 28° than at 16° C., but that the production of conidia was favored by cool temperatures.

Sori formed at 15° and 20° C. (Fig. 28)

In the present studies, horseradish plants were inoculated and incubated in the greenhouse until chlorotic areas appeared on the leaves. The plants were then placed in constant-temperature chambers. The greatest intensity of sori production, the largest sori, and the greatest production of secondary sori occurred at 20° C. (Table 6 and Fig. 28). The results at 15° were as good as at 20° C. except that there

Table 6. — Effect of Temperature During Sori Formation on the Intensity of Sori Production, on the Size of Sori, and on the Intensity of Production of Secondary Sori

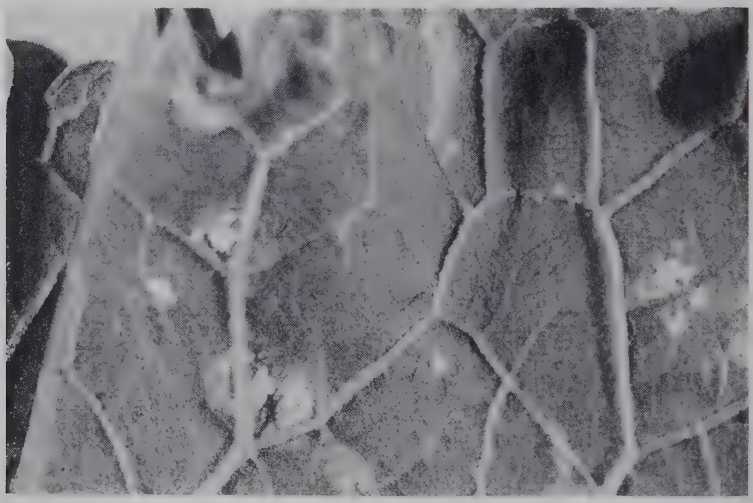
Temperature	Intensity of sori production[a]	Range in size of sori (mm.)	Intensity of production of secondary sori[b]
10° C.	3	.5-6	1
15° C.	5	3.0-10	2
20° C.	5	3.0-10	3
25° C.	3	2.0-8	1
28° C.	2	.5-6	([e])
30° C.	1	.5-5	([e])
35° C.	([d])	([d])	([d])

[a] 1 = sparse; 2 = slight; 3 = moderate; 4 = abundant; and 5 = very abundant.
[b] 1 = slight; 2 = moderate; 3 = abundant.
[c] No secondary sori were formed at this temperature.
[d] No sori were formed at this temperature.

were fewer secondary sori formed. The production of sori was moderate at both 10° and 25° C., but the sori were smaller at 10° C. (Fig. 29) than at 25° C. Very few secondary sori appeared at either of these temperatures, which suggests that the mycelia in the leaf did not continue to spread. The fungus continued to survive, however, since it readily produced sori if the plants were transferred to the 20° C. constant-temperature chamber. At 28° C., sori production was slight and the size of the sori was comparable to the size of the sori formed at 10° C. At 28° and 30° C. the fungus usually died following dispersal of the conidia (Fig. 30).

Age of leaves

The susceptibility to infection of leaves of different ages was investigated. Leaves of greenhouse-grown plants were marked as soon as they emerged, and the plants allowed to continue producing leaves until the first leaves were 12 to 14 weeks old. Leaves of different ages were then inoculated by placing a drop of zoospore suspension (15-25 zoospores per drop) at each of 10 loci on the underside of each leaf. These areas were covered with a thin layer of moist cotton. The plants were next placed in a moist chamber for various periods of time (4, 8, 12, and 24 hours) and then moved to a greenhouse bench. Five such experiments were conducted over a two-year period and



Sori formed at 10° C. (Fig. 29)

Sori formed at 30° C. Note the necrotic area surrounding the small, rather flat sori. (Fig. 30)

involved a total of 260 plants. The number of sori per leaf increased with continued exposure to moisture but was independent of the age of the leaves. However, senescent leaves, in contrast to young, actively growing leaves, gave rise to only very small sori (0.5 mm. to 3 mm. in diameter) and rarely showed a secondary development of sori.

Other crucifers affected

Conidia obtained from infected horseradish plants failed in four different tests to infect either young or old plants of the following species of crucifers: *Capsella bursa-pastoris* (L.) Medic. (see page 45); *Brassica oleracea* L. var. *botrytis* L. (broccoli, var. Green Sprouting); *B. oleracea* var. *gemmifera* DC. (Brussel sprouts, var. Long Island Improved); *B. campestris* L. var. *napobrassica* (L.) DC. (rutabaga, var. American Purple Top; *B. rapa* L. (turnip, var. Purple Top White Globe); *B. japonica Hort.* (leaf mustard, var. Southern Giant Curled); *B. oleracea* var. *capitata* L. (cabbage, vars. Large Late Flat Dutch, Copenhagen Market, Stein's Flat Dutch, Hollander, Early Jersey Wakefield); *Raphanus sativus* L. (garden radish, vars. Icicle, White Strasburg, Early Scarlet Globe, French Breakfast, Early Scar-

let White Tipped); and *B. pekinensis* (Lour.) Rupr. (Chinese cabbage or pe-tsai). Infection was secured only on two-week-old seedlings of *B. oleracea* var. *botrytis* (cauliflower, var. Snowball X); older plants were not tested. Only a few sori were produced on the cotyledons of cauliflower and they were very small, averaging 0.5 mm. in diameter. These limited studies indicate that the physiologic race or races of *A. candida* on horseradish are quite limited in their suscept range. A similar restriction in the suscept range of the race of *A. candida* occurring on radish was reported by Melhus (10) in the United States and by Hiura (6) in Japan.

OVERWINTERING OF THE PATHOGEN

As was noted by Kadow and Anderson (9), one of the most perplexing problems connected with the life cycle of the horseradish white-rust pathogen is its method of overwintering. Various workers (2, 9, 10, 11) have contributed to the knowledge of its life history, but the problem of overwintering has remained controversial. Conceivably, *Albugo candida* could overwinter as conidia, as oospores, or as mycelia within the primary or the set root of horseradish or within other cruciferous hosts. Each of these possibilities was studied in this investigation.

As conidia

In general, experiments have shown that conidia of *Albugo candida* are short-lived. DeBary (2) reported that conidia of *A. candida* survived a maximum period of six weeks, and Melhus (10) found that one good frost would kill most of the conidia of this species.

Outdoors. Two types of experiments were conducted to determine whether conidia of *A. candida* from horseradish are able to overwinter in Illinois. In the first, infected leaves were collected in October from greenhouse and field plants at Urbana and left outside during the winters of 1951 and 1952 in the following ways: (1) on the surface of the soil in an uncovered cold frame, (2) on the surface of the soil and covered with either a wire screen, a burlap bag, or a wooden box with slits, or (3) in open fruit jars covered with two layers of cheesecloth and buried at depths of six to twelve inches. Conidia were scraped from the leaves in the second week of April of each of the following years and tested for viability at 15° C. for 48 hours. No germination resulted from any of the trials, and such conidia failed to infect horseradish plants placed in a moist chamber for 48 hours.

Refrigeration. In the second type of experiment, conidia were stored in various ways in a refrigerated room regulated at 0.5° to 3° C. Detached conidia that had been scraped from infected leaves and placed in corked vials did not survive 2 weeks of storage. Conidia from sori-bearing leaves which had been cut into small pieces and placed in corked vials gave 5 to 15 percent germination at the end of 2 weeks but none at the end of 4. Conidia from whole, detached leaves that had been placed in plastic bags for storage survived two weeks and caused a trace of infection when inoculated on healthy plants. They neither germinated nor caused infection two weeks later. Conidia from whole, detached leaves stored on open shelves were tested every 2 weeks for 10 weeks. At the end of 8 weeks only 6 percent of them germinated and they caused only a trace infection. They did not germinate or cause infection at 10 weeks. The longest storage survival was by conidia on the leaves of potted plants likewise held at 0.5° to 3° C. When the plants were watered throughout the test period, the conidia survived for as long as 10 weeks and retained their infectivity. On plants that were not watered, the conidia survived for 3 to 4 weeks. These results demonstrate that conidia of *Albugo candida* are short lived and are very likely unable to survive winters in Illinois, thus confirming the work of earlier investigators.

As oospores

Oospores are produced most often under conditions apparently unfavorable for the pathogen, such as when the suscept begins to decline in vigor. Oospore formation by *Albugo candida* in suscept tissue appears to depend somewhat upon the particular plant species affected (1, 15, 21). Although Wilson (21) in 1907 reported examining oospores of *A. candida* in horseradish leaves, Kadow and Anderson (9) were unable to find oospores in "old plant material of horseradish." They comment that "So far as is known, oospores are not formed on this host and if formed are probably so rare as to be of little importance as a source of spring infections." In 1952, Takeshita (Endo) *et al.* (17) reported finding abundant oospores in the leaves of the common variety of horseradish. Oospores were obtained once following the immersion of the leaves of greenhouse-grown plants in a heavy suspension of conidia for 24 hours. They did not appear in repetitions of the experiment. However, the fact that oospores had been produced in abundance at all made it important to determine whether oospores are formed under field conditions and, if so, in sufficient quantity to be a factor in the overwintering of the fungus.

In commercial fields. Periodic surveys of commercial horseradish fields were made in 1952 and 1953 in order to obtain information on the occurrence and prevalence of oospores, of systemically infected plants, and of other factors relating to the overwintering of the pathogen. In 1952, twelve fields located in the vicinity of Des Plaines, Illinois, were surveyed at 10- to 14-day intervals. In 1953, three fields near Collinsville, Illinois, were surveyed every 10 to 20 days, and three fields near Des Plaines every 4 to 5 weeks. In addition, from 1951 through 1953, systemically infected plants in the Urbana experimental plots were checked every 7 to 10 days for oospore development since several investigators (1, 15, 21) working with other species of Albugo had reported readily finding oospores in suscept plants.

Oospores were found for the first time in commercial fields in very limited numbers in late September, 1952. Although subsequent examination in October and November revealed a definite increase in numbers of antheridia and oogonia, no increase in the number of oospores was observed. The advent of cold weather probably retarded further oospore formation. Oospores were found most frequently in systemically or locally infected petioles, though they occurred in only about 10 percent of such petioles sampled and the number was extremely low. The crown region of systemically infected roots rarely contained oospores (Fig. 11).

Association with fernlike leaves and resistant varieties. In 1953 as in 1952, the same phenomenon was noted with respect to oospore development in field-grown plants with, however, one notable exception. In early June, oospores were found in limited numbers in the petioles of systemically infected plants at Urbana but not in commercial fields. The Urbana planting consisted of plants which had overwintered in the field, and nearly all of these plants had produced "cut-leaves" and seed stalks in the spring. On the other hand, all plants in commercial fields were in the vegetative stage since they had grown from set roots planted in the spring. In 1953 oogonia and antheridia were not found in plants in commercial fields until October when the plants were in the cut-leaf stage. These organs were found in fair abundance in local infections occurring in the leaves, but, as in 1952, very few oospores developed.

Natural petiole infection sometimes occurred on plants of the common variety growing in the greenhouse during the fall and winter. In 1953 such petioles were marked and observed at various times for the presence of oospores. As the leaves of these plants changed from broad (vegetative stage) to cut-leaf (reproductive stage), oospore

formation increased. The time usually required for oospore formation was also shortened from 4 to 5 weeks to 2 to 3 weeks. The association between oospore formation and the cut-leaf stage was confirmed when 20 plants of the common variety with cut leaves and 20 plants with entire leaves were inoculated in the greenhouse during the winters of 1953 and 1954. A similar increase in oospore production of *Albugo occidentalis* in spinach plants in the reproductive stage over those in the vegetative stage was reported by Raabe and Pound (16).

The production of oospores during the cut-leaf stage was also observed in the Bohemian variety of horseradish, which is resistant to white rust, and in certain progeny of the crosses between the Bohemian and the common variety. Oospores were often found in the hypertrophied petioles of some of the resistant hybrid plants. Ten of these plants were uprooted, and 5 cuttings were made from each root and planted. These plants, as well as clones or cuttings of the common variety and of hybrids with no apparent resistance to white rust, were inoculated by the leaf immersion method. Although some variation in oospore formation occurred among the clones, oospores tended to form most often in plants resistant to the pathogen. Occasionally, oospores were found in the very susceptible clones of the common variety, but they were never as abundant as in the resistant clones. This experiment was repeated with essentially the same results.

As mycelia

In other suscepts. Overwintering of various species of Albugo by means of mycelia in suscept plants is probably not uncommon. Melhus (11) showed clearly that *A. candida* could overwinter as mycelia in the winter annual *Capsella bursa-pastoris* — shepherd's purse — and that the fungus resumes its activity in the suscept the following spring with the return of favorable environmental conditions. Hence infected shepherd's purse plants surviving the winter in this manner might serve as sources of primary inoculum for horseradish plants. However, according to Kadow and Anderson (9), Duis, a former graduate student at the University of Illinois, found that the form of *A. candida* on horseradish infects only a few of the other crucifers and that conidia taken from common cruciferous weed suscepts will not infect horseradish. In the present investigation, cross-inoculation experiments were conducted with conidia obtained from horseradish and shepherd's purse. These experiments were repeated eleven times during the springs of 1952 and 1953, but successful cross-infection was not obtained.

Infection in volunteer plants. Kadow and Anderson (9) reported that mycelia of *A. candida* were able to overwinter in discarded horseradish crowns, but they were unable to find the mycelia in the primary and set roots. Mycelial infection in a root may reveal itself in the form of systemically infected shoots which grow from the infected area. Therefore the extent to which mycelia survive the winter in infected roots may be approximately determined by observing the number of volunteers on which the fungus appears shortly after shoot emergence begins in the spring. In order to determine the role of discarded roots or portions thereof in the overwintering of the fungus, field surveys were conducted in the spring of 1952 and 1953 to look for systemically infected plants. In the East St. Louis area, where sweet corn is often planted the year following horseradish, spring surveys of nineteen sweet corn fields that had been planted to horseradish the previous year revealed many volunteer horseradish plants but a total of only six systemically infected volunteer plants. These fields had been cultivated at least twice before the survey was conducted.

The effect of cultivation on the development of systemically infected volunteers was tested at Urbana. Two identical plots, consisting of approximately 1,200 plants each, were surveyed at regular intervals during 1951 for systemic infection. Infection was as severe in one field as in the other, affecting about 130 plants in each. The plants in one plot were plowed under in late October and the plot was deep disked twice in the early spring of 1952. The plants in the second plot were left to overwinter. Though many volunteer plants appeared in the plowed and disked plots in 1952, not a single systemically infected plant was observed. In the undisturbed field, 89 out of the total of 131 systemically infected plants observed during 1951 produced infected shoots in 1952, thus showing that cultivating prevents infected volunteers from appearing and that systemically infected plants are common among undisturbed plants.

Cull piles were also inspected during the spring for the presence of systemically infected plants. Here, from 1 to 20 systemically infected plants were found in 14 out of 18 cull piles examined during the 1952-1953 surveys. Such plants were most abundant in May and June and could have served as sources of inoculum in the form of airborne conidia, although there was no pattern of spread to indicate that this had occurred.

Infection in sets. Surveys of thirteen commercial horseradish fields and the experimental plot at Urbana indicated that white-rust mycelia overwinter in set roots saved for planting as well as in roots left in the

field or in cull piles. Of the number of sets examined before planting in 1952, an average of 1 set root out of 1,134 was found to be infected. In 1953 the average was 1 out of 603. A half-acre field examined in 1952 had the unusually high incidence of 1 out of 233, and the Urbana experimental plot examined in 1953 had 1 out of 67. A ratio such as 1 to 1,134 must not be considered insignificant since a single infected plant furnishes enough inoculum for a large field. A further check on the possibility that some infected sets are planted was furnished by the fact that systemically infected roots were found in commercial fields early in the spring in such an advanced stage of disease that it is extremely unlikely that infection had occurred during the current season.

Types of systemic infection. When set roots systemically infected with mycelia are planted, infection may occur in some of the emerging shoots. However, there was cause to doubt that every case of systemic shoot infection arose from mycelia that had overwintered in sets, since systemic infection was found in most commerical fields at other times than just in the spring.

Three types of systemic shoot infection were distinguished. In the first type the infected shoots arose from or near the crown in the spring (Fig. 8). The infection in those that were found within six weeks of the start of shoot emergence probably originated from mycelia that had overwintered in the crowns of the sets just planted. The second type consisted of systemically infected shoots which originated at a distance of usually 3 inches or more from the crown (Figs. 6, 7, 15). These shoots, which were extremely fasciculated, grew from hypertrophied areas of the root. Occasionally such shoots appeared within six weeks of shoot emergence. The third type of systemic infection was found in very small leaves (Fig. 5) in late September or October and may have been caused by airborne conidia. In some instances the root was not infected, or only slightly.

Systemically infected roots from the Urbana plots (page 46) were harvested in the fall of 1952 and were classed into two types depending upon the location of the systemically infected shoots: Type 1 gave rise to shoots originating from or near the crown and Type 2 to shoots originating at a distance from the crown. These types were studied to determine their relative frequency and to determine what factors influenced their origin and development. Each type of root was either photographed or a drawing was made showing the location of the systemically infected shoots, the number and location of set roots, and their distance from the crown. Root dimensions were also taken. Be-

ginning at the crown, each primary root was cut into alternate 2½- and ⅛-inch sections. Secondary or set roots were cut in identical fashion, beginning at their point of attachment to the primary root. The 2½-inch sections were washed thoroughly in several changes of tap water, disinfected in a 1:10 Chlorox-water solution, rinsed thoroughly in several changes of tap water, and planted in sterile soil in pots. The shoots and leaves developing from these sections were observed in the greenhouse for the development of white rust. Whenever rot prevented shoot emergence or whenever white rust developed on the shoots, the corresponding ⅛-inch sections which had been fixed in formalin-acetic acid-alcohol were examined under the microscope for the haustoria and mycelia typical of *A. candida.* Examination of 262 Type 1 roots showed that the extent of mycelial invasion down into the root was inversely proportionate to the diameter of the root (Table 7). This showed that the fungus is not confined to the crown of the horseradish plant but may grow into any portion of the primary or secondary roots; however, the roots over an inch in diameter were seldom invaded for a distance of more than 5 inches and usually less.

An additional 248 badly decayed systemically infected primary roots (Type 1) were also studied. The mycelia were found by microscopic examination to extend at least 10 inches down the primary root in 4 cases.

A total of 183 Type 2 roots were examined and 110 were found to have mycelial infection extending nearly into the crown of the primary root. In order to restrict the examination to those roots where the infection did not appear to have started at or near the primary root crown, only the remaining 73 roots were sectioned and treated as the

Table 7. — Relation Between the Diameter of the Primary Root and the Depth of Infection Following Crown Infection: Sets Planted in the Spring of 1951 and Allowed to Overwinter in the Field; Roots Examined in the Fall of 1952

Diameter of primary root, inches	Number of roots examined[a]	Linear depth of invasion of roots, inches					
		0-2.5	2.6-5.0	5.1-7.5	7.6-9.0	9.1-11.5	11.6-14.0
		(percent)					
.5-1.0	47	100	55	38	11	6	4
1.0-2.0	162	100	22	6	1	.6	.6
2.0-3.0	53	100	9	0	0	0	0

[a] Only infected roots were examined.

Type 1 roots had been. This examination revealed that in 57 of these 73 roots there was no evidence of infection between the crown and the infected area from which the secondary shoots grew (Fig. 7).

The same method of examination — planting 2½-inch sections of the infected root and examining ⅛-inch sections under the microscope when the larger sections gave evidence of infection — was applied to 18 obviously infected roots discovered among 1,200 sets examined prior to planting at Urbana in 1953. In 8 of these 18 roots, the infection was remote from the crown and did not extend near it.

These 8 sets as well as the 57 primary roots just mentioned illustrate that not all set infection nor all primary root infection originates in the crown or is contiguous to that which does, although, in fact, systemically infected shoots, like healthy shoots, usually do develop from the crown during the spring. As oospores were rarely found in commercial fields, whatever set infection was not initiated by the spread of mycelia within the primary roots probably was initiated by conidia.

Inoculation of set roots. Experiments were next conducted to determine whether conidia can infect set roots. It was found that systemic crown infection could readily be obtained on horseradish plants by wetting very young leaves with a suspension of conidia. Inoculating older leaves resulted in the infection of the leaf blades and petioles but not of the roots. If the plants had been thoroughly watered before inoculation, it was not necessary to put them in a moist chamber. However, systemic infection could not be obtained in all of the crowns unless a high relative humidity was maintained by putting the plants in a moist chamber. The time required for the development of systemic infection varied from 18 to 41 days.

A microscopic examination of young inoculated leaf buds, cleared by boiling them in a saturated solution of chloral hydrate and crystal violet or by placing them in pyridoxine, revealed that the zoospore germ tubes entered through the stomata of the sets. As soon as this entrance is accomplished, the life cycle begins again and systemic, intercellular hyphae develop in both the root tissue and the shoot.

To determine whether the entrance of germ tubes into the stomata of young buds was the only way infection could occur, the following experiment was performed. Sets were divided into three groups: sets with buds just appearing, budless sets that were not wounded, and budless sets that had been wounded with a sterile needle. Sets from each group were planted in sterile soil, in nonsterile soil, and in sterile sand and covered to a depth of 1½ inches. The crowns of half the

sets in each group had been dipped in a 500,000 per cc. suspension of conidia before planting. The same heavy suspension was poured on the soil or sand over the other half after those sets were buried. Systemic infection occurred only in the sets that possessed buds. All the budding sets which had been dipped in inoculum were systemically infected regardless of which medium they were planted in. Of the budding sets that had been buried before the conidia were poured over them, 6 out of the 36 sets buried in sand became systemically infected, but none of the sets buried in either kind of soil. To check these results, three more groups of sets with buds and wounded or unwounded sets without buds were placed in plastic bags or in a moist chamber after being dipped in a suspension of zoospores. A temperature of 15° C. was maintained during incubation. Again, systemic infection occurred only in the set roots that possessed buds.

These results indicate that systemic infection of set roots possessing buds occurs readily. Failure of such roots to become infected systemically when a suspension of conidia was poured over the soil after planting can be attributed to failure of the conidia to reach the buds rather than to inhibition of germination by soil constituents. This supposition is supported by the fact that neither 10 grams of sterile nor of nonsterile soil added to 200 cc. of a heavy suspension of conidia adversely affected zoospore emergence, zoospore germination, or the elongation of germ tubes.

DISCUSSION

The development of epiphytotics (epidemics) of white rust, if inoculum is present, requires an abundance of moisture, as dew or rain, together with cool or mild temperatures. Germination and disease development are both favored most by the range of 15° to 20° C. Germination is also very good at 10° C. and disease development at 25° C., but germination is much slower at 10° C. than at 15° or 20° C. It has been observed frequently in Illinois that epiphytotics occur during or following moist, cool weather and that the disease is considerably less damaging during hot weather. Thus the disease may be severe from the time of plant emergence in the spring until July 15. However, it ordinarily causes little damage during July and August. It may then reappear the first week of September and continue to spread and cause damage until killing frost. However, if temperatures in July and August are below normal or if heavy dews prevail, white rust may develop and spread without interruption.

The germination of conidia requires the presence of free water and is influenced by relatively small differences in temperature and by the age of the sori from which the conidia come. Conidia cannot sustain their viability by means of moisture in the air. An exposure to even 100 percent relative humidity for a few hours markedly lessens the percentage of conidia that germinate. A 24-hour exposure to 100 percent relative humidity causes abnormal germ tube growth from the zoospores that are released. Zoospore release is fastest at 15° C., zoospore germination is practically identical at 15° and 20° C. for the first nine hours, and germ tube growth is fastest at 20° C. While these temperature ranges are not extremely critical, yet it is evident that not all phases of germination proceed with equal dispatch at the same temperature. If the total process of conidial germination is held at a single temperature until it achieves germ tubes capable of entrance, the temperature at which this occurs the soonest, both in distilled water and on the leaf, is 20° C.

Leaf desiccation does not appreciably increase germination, and chilling the conidia at 10° C. helps germination only insofar as it provides a favorable temperature for zoospore release before the temperature is raised to an optimum for zoospore germination. Maturation of conidia is delayed below 10° C. and is accelerated above 25° C. The conidia of sori developed at 30° C. become senescent before they are released. Under field conditions, however, probably most of the conidia are mature by the time the sorus ruptures. Several investigators who have worked with *Albugo candida* have complained of the erratic germination of conidia collected from greenhouse-grown plants. Probably the principal cause was the differences in the ages of the sori from which the conidia were taken.

Surveys of commercial horseradish fields in Illinois show that the white-rust pathogen is introduced with the sets in each planting. While the percentage of infected sets planted in each field is quite small and varies from year to year, the systemically infected plants arising from these sets provide most of the primary inoculum for the field or region. Systemically infected plants growing from horseradish cull piles and in fields planted to horseradish the preceding year also are potential sources of inoculum. There is no evidence that oospores play a major role in the overwintering of the pathogen.

Infection of set roots may result from (a) the mycelia growing from the crown down through the primary root to the set (or secondary) root or (b) zoospore germ tubes entering the stomata of leaf buds on the set. Complete systemic invasion through the length of the primary root is rarely found in the field because such infected roots

are soon destroyed by secondary rot-producing bacteria and fungi. On the other hand, it is relatively easy to obtain localized white-rust infections on a set with buds by inoculating it with a suspension of conidia. In the field the conidia from sori on the leaves might be washed down through the soil to the roots by rains prior to harvest or could easily fall on the sets during harvest.

During storage the sets are exposed to nearly ideal conditions for infection. The sets, after being broken off the primary root, are placed in cool, damp storage rooms or are buried in trenches out-of-doors. The temperature then is usually below 15° C. and storage conditions do not change abruptly. At 10° C., when there is sufficient time, the conidia form well in the sori and achieve a high maximum percentage of germination. The sets are exposed to light for varying periods of time after harvest and even in storage, which stimulates the formation of leaf buds. Furthermore, the practice of piling roots together for storage and then wetting the pile to lower the temperature provides excellent conditions for the germination of conidia and for subsequent leaf bud infection. Sometimes, too, the primary roots are stored with the sets still attached before being sold. If leaf infection has been heavy, this undoubtedly increases the amount of inoculum present because the crowns of the primary roots are exposed to conidia in the field. It is apparent, however, from the small proportion of sets that were found to be infected before planting, that the spread of infection during storage is not comparable to the spread of infection in the field.

There are certain exclusionary and eradicatory measures based on the results of this investigation that should be followed if white rust is to be controlled:

1. Sets for planting the following year should be taken only from the terminal end of the primary root and should be removed from the primary root before being placed in storage. Those sets located near the crown and not detached in lifting showed the highest frequency of white-rust infection.

2. Sets that show discolored, swollen, or cracked areas at planting time should be destroyed.

3. All horseradish cull piles should be sprayed with kerosene and destroyed by burning before the new crop is planted.

4. Fields planted to horseradish the preceding year should be cultivated early, frequently, and thoroughly to eradicate systemically infected volunteer plants.

5. All systemically infected plants and attached roots should be pulled up, removed from the field, and destroyed as soon as they emerge

in the spring. This will require inspection at least once every 10 to 14 days throughout the spring and perhaps during the summer.

6. The foliage of horseradish plants should be destroyed at least 7 days before harvest with a "Rotobeater" or similar machine, thus lessening the danger of conidia falling from the leaves onto the roots during harvest. It is likely that most set infection is initiated by conidia falling on the terminal roots at this time.

Protective fungicides for white-rust control will be most valuable if applied from shortly after shoot emergence in the spring until around July 15 and again from September 1 until killing frost. However, if abnormally low temperatures or heavy dews prevail during July and August, applications of fungicides may be needed throughout the entire growing season.

SUMMARY

Studies were conducted on the white-rust disease of horseradish and the causal fungus, *Albugo candida,* in order to obtain information on the factors that influence the germination of the fungus and the development of the disease. Information was sought also on the methods by which the fungus overwinters.

Conidia almost invariably germinated indirectly by releasing 5 to 8 zoospores which in turn produced germ tubes capable of entering the stomata of the horseradish plant. Both zoospore release and germ tube production were determined in germination studies.

Germination of conidia took place only in free water. Germination was essentially the same in rain, distilled, double-distilled, and resin-exchange-treated water but was considerably poorer in tap water. Germination was not affected by concentrations of conidia up to 500,000 per cc. No consistent difference in germination was found between conidia exposed to light and those kept in the dark. Conidia germinated over a pH range of 3.5 to 9.5 with the optimum about 6.5. Temperature influenced the rate of development of conidia in the sori. Conidia that were developed in the sori at 25° C. attained maximum germination when the sori were 5 days old; at 20° C. at 6 days; at 15° C. at 7 days; and at 10° C. at 10 days. Conidia incubated at 30° C. reached maturity in 3 days but had the poorest germination. Desiccation of conidia was found to be neither necessary nor favorable for their germination. Holding conidia in water at 10° C. before placing them at higher temperatures did not improve their germination except in some cases where maximum zoospore release had been attained before the transfer was made.

The rate and percentage of the germination processes were each determined in distilled water. The optimum temperature for maximum zoospore release from conidia was 15° C.; for zoospore germination, 15° C.; and for germ tube elongation, 20° C. Zoospore release occurred most rapidly at 15° C., beginning in slightly less than 1 hour. Zoospore germination was equally rapid at 15° and at 20° C. for the first 9 hours. Germ tube growth was fastest at 20° C. Temperatures of 25° C. or above during germination markedly reduced both the rate and percentage of release. Conidia that had been exposed to 35° C. for 12 hours while still in the sori did not germinate well. Zoospores rapidly lost motility at 15° C. or above and retained it the longest at 5° to 10° C.

Dusting plants with a conidia-talc mixture was not as effective as spraying them with inoculum or immersing the leaves in inoculum. After inoculation, intermittent atomization with water caused a lower percentage of infected plants and a lesser intensity of infection than did continuous atomization with water or continued immersion in a suspension of conidia. The maximum intensity of infection was obtained by immersing the leaves for 18 hours in a suspension of conidia at 20° C. A trace of infection followed only 3 hours of immersion at this temperature.

The length of time required for chlorotic areas to appear after inoculation, varied from 5 to 7 days at 28° C. to 16 to 20 days at 10° C. Sori were visible within 1 to 3 additional days, depending on the temperature.

The largest number of sori and conidia, and the most rapid development of secondary sori occurred on plants held at 20° C., followed closely by plants held at 15° C. All phases of development were curtailed below 10° and above 25° C. The pathogen usually died out at 28° C. or above following dispersal of the conidia.

Young leaves and leaves 12 to 14 weeks old were equally susceptible to infection. However, the sori on the older leaves were considerably smaller than those on the young leaves and the development of secondary sori was rare.

The fungus could not be cross-inoculated from horseradish to other crucifers except very young cauliflower seedlings.

Oospores of the pathogen are produced on rare occasions in the leaf blades and petioles but are not considered to be important in the overwintering of the pathogen. There is evidence that oospore production is initiated with a change in the plant from the vegetative to the reproductive stage of growth.

Systemically infected plants were found in the spring in almost all commercial horseradish fields, in uncultivated fields planted to horseradish the preceding year, and in cull piles. These plants, though few in number, serve as sources of inoculum for entire fields.

The pathogen overwinters principally as mycelia in the set (secondary) roots that are saved each fall for planting the following spring. Mycelia grow occasionally from the infected crown of the plant down through the primary root into the sets. The length of linear infection of the primary root is inversely proportionate to the root's diameter. Mycelial infection of sets is probably initiated in most cases by conidia falling on the terminal roots at harvest.

Infection of the small leaf buds of sets was obtained by dipping them in a suspension of conidia. Systemically infected shoots and roots developed from the inoculated buds.

On the basis of this investigation, several control measures for white rust are recommended. These include (a) taking sets only from the terminal end of the primary root and immediately separating the sets from the primary roots as soon as they are harvested, (b) elimination before planting of all sets showing discolored, swollen, or cracked lesions, (c) immediate eradication of plants showing systemic infection in the spring and summer, and (d) killing horseradish foliage by mechanical means at least seven days before harvest to lessen the possibility of viable conidia falling onto the roots before they are placed in storage.

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